Uliano et al. Nutrire (2016) 41:20
DOI 10.1186/s41110-016-0024-8

Nutrire

RESEARCH Open Access

Association between paraoxonase 1 (PON1) enzyme activity, PON1 C(−107)T polymorphism, nutritional status, and lipid profile in children

Gabriela Uliano[1], Ludmila C. Muniz[1], Carlos C. Barros[1], Augusto Schneider[1] and Sandra C. Valle[1,2,3*]

Abstract

Background: Paraoxonase 1 (PON1) is an enzyme that possesses anti-atherogenic and anti-inflammatory properties with serum levels determined by genetic and exogenous factors. Lower serum PON1 arylesterase activity is associated to metabolic alterations related to childhood overweight and onset and/or development of diabetes and CVD later in life. However, data on the relationship between genetic PON1 polymorphisms and nutritional status as well as lipid profile in children are limited.
To investigate the distribution of the C(−107)T PON1 gene polymorphism and its relation with serum PON1 enzyme activity, nutritional status and lipid profile in children.

Methods: A cross-sectional study was performed including 73 children aged 5 to 7 years who attended public pediatric clinics. PON1 C(−107)T, arylesterase activity, body mass index for the age, and serum lipid profile were evaluated.

Results: PON1 activity was higher in overweight children compared to the normal weight ones ($p = 0.02$). The genotypic frequency did not differ between the two groups ($p > 0.05$). Carriers of *CC* genotype had higher enzyme activity than *T* allele carriers, and this difference was greater among normal weight children. HDL levels were higher among normal weight children carrying CC genotype, compared to those carrying the T allele ($p < 0.01$).

Conclusion: The PON1 C(−107)T polymorphism is associated with higher serum enzyme activity in children, as observed previously in adults. In addition, this polymorphism also shows association to higher high density lipoprotein (HDL) levels and serum PON1 arylesterase activity in the normal weight children studied.

Keywords: Arylesterase, Nutritional status, Childhood, SNP, Overweight, Lipid profile

Background

Human paraoxonase-1 (PON1) is a calcium-dependent esterase, synthesized primarily in the liver, which circulates in the bloodstream associated to high-density lipoprotein (HDL) [1]. Originally, the PON1 was identified for hydrolyzing organophosphates, and its name refers to the enzyme's ability to degrade paraoxon (paraoxonase activity) [1]. In addition, the enzyme degrades lipophilic lactones (lactonase activity), phenylacetate (arylesterase activity), and nerve agents [1]. Studies reported that PON1 plays a significant antioxidant activity of HDL by protecting low-density lipoproteins (LDL) to lipid peroxidation and, thereby, attenuates the development of atherosclerosis [1–3]. Moreover, PON1 modulates the anti-inflammatory role of HDL and exerts a defensive effect against atherogenic changes, like homocysteinylation of HDL and LDL [1]. Finally, increased serum PON1 activities are associated to the reduction of endothelial damage and cardiovascular disease (CVD) risk [2, 3].

Several polymorphisms in the coding and promoter regions of the PON1 gene (including $PON1_{192}$, $PON1_{55}$, $PON1_{-162}$, $PON1_{-832}$, $PON1_{-909}$, $PON1_{-1076}$,

* Correspondence: sandra.valle@ufpel.edu.br
[1]Department of Nutrition, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil
[2]Food for Health Science Centre, Lund University, Lund, Sweden
Full list of author information is available at the end of the article

and PON1$_{-1741}$) have been associated with changes in the enzyme's activity and/or concentration [1, 4, 5]. Among the genetic changes, the single nucleotide polymorphism (SNP) identified in the position −107 in promoter region of the PON1 gene exerts significant effects on serum activity [6, 7]. It contributes with up to 25% of the variations in the PON1 expression in Caucasian adults, and the presence of the C allele results in PON1 levels up to twice higher than for carriers of the T allele [6, 8, 9]. The PON1 T(−107)C SNP accounts for approximately 12% of the serum activity variability between individuals, contributing more than other SNP, even in combination with exogenous factors [10]. For that reason, we choose this SNP for the current study. A number of SNP affect the enzyme catalytic efficiency for some substrates such as paraoxon [10]. Therefore, the use of phenyl acetate provides a better indication of the enzyme activity in the serum [10].

A two- or threefold rise in childhood overweight has been identified in recent years in developed and in developing countries [11]. The childhood overweight and overweight-related issues, such as obesity, dyslipidemia, glucose intolerance, and hypertension, are important subjects of debate in children's health at this moment [5, 11]. These conditions have been considered as risk factors leading to CVD untimely [5]. However, few studies have analyzed the activity of PON1 in children and its association with weight and risk factors for CVD. In particular, one study performed aimed to genotype and identify the frequency of PON1 C(−107)T in a children population and observed that the frequency of both alleles was very similar [9]. Moreover, it has verified that the PON1 arylesterase activity was significantly higher in children homozygous for the C allele [9].

The serum PON1 activity also is affected by a number of exogenous factors such as nutritional and health status [7, 10]. In a recent study, children, 12 year-olds, with body mass index (BMI) > 95th percentile had significantly lower PON1 levels in comparison to normal weight [12]. In addition, lower serum PON1 lactonase and arylesterase activities are associated to metabolic alterations related to childhood overweight and onset and/or development of diabetes and CVD later in life [1, 5]. Nevertheless, data on the relationship between PON1 and nutritional status as well as lipid profile in childhood are limited and sometimes controversial, mainly due to differences in measurement techniques and data analysis [13, 14]. Such situation is expected to be clarified by the analysis of the interaction between gene, PON1 arylesterase activity, and nutritional status. The present study aims to investigate the distribution of the C(−107)T PON1 gene polymorphism and its relation with serum PON1 enzyme activity, nutritional status and lipid profile in a population of children assisted by pediatric clinics of two cities in southern Brazil.

Methods

Study population

The sample of this study is part of a cross-sectional study on the activity of PON1 and its associated factors in children aged 5–7 years old, from Rio Grande do Sul (RS), Brazil. The study was conducted from January to October 2014 with children attending the pediatric clinic of the College of Medicine/Universidade Federal de Pelotas (UFPEL) in Pelotas–RS, Brazil and the pediatric clinic of Pinheiro Machado–RS, Brazil. Children diagnosed with liver diseases, cerebral palsy, bone dysplasia or neoplasia, and those with special needs (physical or motor) and with genetic alterations such as Down syndrome and thalassemia were excluded.

All children referred to pediatric clinics during data collection were assessed, being invited to participate in the study are those who did not have any exclusion criteria. Information on the age and sex of the child were collected from medical records, anthropometric measurements were performed, and children were referred for blood collection in a clinical laboratory.

Ethics statement

All procedures followed were in accordance with the ethical standards, and study protocols were approved by the Ethics Committee on Human Research of College of Medicine/Universidade Federal de Pelotas (Report number: 654.439). Consent was obtained at the time of enrollment by the study staff. The parents or guardians of the children were informed concerning the purpose and procedures of the study before written consent was obtained. The child's consent was obtained orally before the beginning of the evaluations.

Anthropometric measurements

Weight and height data were measured using a digital platform scale (Welmy®) with 150 kg capacity and 100 g accuracy, and an attached stadiometer with 200 cm capacity and 0.5 cm precision. Anthropometric measurements were taken twice by trained interviewers, being the average of the two measures used in the analysis. To assess the nutritional status, the body mass index (BMI) for age in z score was used, as recommended by the World Health Organization, 2007, through the Anthro-Plus program [15]. Children with BMI-for-age> + 1SD were classified as overweight.

Biochemical analyses

After a 12-hour fasting period, 7-ml blood samples were collected in the clinical laboratory, between 8 a.m. and 10 a.m. The samples were centrifuged, and the serum was immediately used for verifying serum lipids. Total cholesterol (TC) (Biosystems, RJ, BR), the cholesterol linked to high-density lipoproteins (HDL-C) (Biosystems, RJ, BR),

and triglycerides (TAG) (Doles, GO, BR) were determined by the enzymatic colorimetric test, following manufacturer's instructions. The cholesterol linked to low-density lipoproteins (LDL-C) was calculated by the Friedewald formula. The reference values considered adequate were those proposed in I guidelines for atherosclerosis prevention in childhood and adolescence, developed by the Brazilian Society of Cardiology [16].

Determination of PON1 arylesterase activity levels

To measure the arylesterase activity of PON1, the serum samples were frozen and kept at −20 °C until further analysis. The arylesterase activity of PON1 was measured in duplicate, using phenyl acetate as substrate. The enzymatic activity was calculated from the rate of formation of phenol by increasing the absorbance at 270 nm, at 25 °C in a spectrophotometer (FEMTO®). Samples were diluted 1:3 in buffer 20 mM Tris/HCl (Sigma Chemical Co., St. Louis, USA), pH 8.0, containing 1 mM of CaCl2 (Vetec Chemical Co., RJ, Br). The reagent solution was composed by the buffer, to which it was added 1 mM of phenyl acetate (Sigma Chemical Co., St. Louis, USA). The reaction was determined after 20 s of retention, and the absorbance was measured for 60 s. It was considered one unit of PON1 arylesterase activity equal to 1 μM phenol/minute, expressed in kU/L, based on the phenol extinction coefficient. Blank samples containing deionized water were used to correct non-enzymatic hydrolysis.

Genotyping

Genomic DNA was extracted from heparinized blood samples according to standard procedures and quantitated using a spectrophotometer. The SNP was determined by polymerase chain reaction (PCR), followed by restriction enzyme digestion (*Bsr*BI), and agarose gel electrophoresis. The amplification by PCR of PON1 C(−107)T was performed using the primers: forward 5′- AGC-TAG-CTG-CGG-ACC-CGG-CGG-GGA-GGA-G -3′ and reverse 5′- GGC-TGC-AGC-CCT-CAC-CAC-AAC-CC -3′. The amplification program included an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 67 °C for 45 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR products were digested at 37 °C for 2 h using *Bsr*BI (New England Bio Labs, Cambridge, UK). The lower case base in PON1 (−107) upstream primer indicates a mismatch, introducing a restriction site for *Bsr*BI enzyme, because there is no specific restriction site cutting the DNA original sequence. After digestion, the C allele was identified by 28 and 212-bp fragments, whereas the T allele resulted in a no-digested 240-bp fragment. DNA fragments were separated by electrophoresis on 3% high-resolution agarose gel (Kasvi, Parana, BR), stained with SYBR Safe (Applied Biosystems).

Statistical analysis

Data were double entered in Microsoft Excel 2010 and analyzed in STATA software, version 12.0 (Stata Corp., College Station, USA). Initially, the normality of the data was tested from the Shapiro-Wilk test. The description of the variables was performed using absolute and relative frequencies for categorical variables. For numeric variables, mean and standard deviation (SD) and median and interquartile range were used (p25–75) in the case of not approximately normal variables. PON1 activity, according to major categorical exposures was assessed by Student's *t* test, Mann–Whitney, or Kruskal–Wallis, depending on the nature of the variables. Allele frequencies were deduced from the genotype distribution. The χ^2 test was used to test the Hardy–Weinberg equilibrium (HWE), with the observed and expected frequencies obtained. The distribution of nutritional status among sex or genotypes was also compared by the χ^2 test. *P* values less than 0.05 were considered to indicate statistical significance.

Results

For the analyses, 73 children were included, composed of 37 males (50.7%). The median PON1 arylesterase activity was 86.5 (66.6 to 103.6 kU/L) , being similar between boys (84.8 ± 24.3 kU/L) and girls (90.2 ± 28.9 kU/L) ($p = 0.50$). Among all evaluated parameters, differences were observed across sex only for TAG ($p = 0.03$). Table 1 shows the main demographic and biochemical characteristics of the studied population, stratified by nutritional status. Of all children evaluated, 41.1% ($n = 30$) were overweight. Overweight children had higher PON1 activity than that of the normal weight group ($p = 0.02$).

The allelic frequency for the PON1 C(−107)T genotype was 60% for the C allele and 40% for T allele. The

Table 1 General characteristics and biochemical laboratory data from normal weight and overweight children aged 5–7, Pelotas, RS, Brazil, 2015. ($n = 73$)

	Normal weight ($n = 43$)	Overweight ($n = 30$)	*P* value
Sex; F:M[≠]	20/23	16/14	0.66
	Mean (SD)	Mean (SD)	
Age (years)[b]	5.9 (0.8)	5.9 (0.8)	>0.99
HDL-C (mg/dL)[b]	52.8 (12.8)	50.0 (15.0)	0.39
LDL-C (mg/dL)[b]	85.1 (30.3)	93.7 (28.8)	0.22
	Median (p25–p75)	Median (p25–p75)	
TC (mg/dL)[a]	162.0 (125.0–179.0)	165.0(137.0–187.0)	0.29
TAG (mg/dL)[a]	60.0(50.0–86.0)	71.5(56.0–84.0)	0.17
PON1 activity (kU/L)[a]	77.6(64.1–96.1)	97.2(78.7–105.5)	0.02

HDL-C high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TC* total cholesterol, *TAG* triglycerides, *PON1* paraoxonase 1
[a]Mann–Whitney test
[b]Student's *t* test
[≠]χ^2 test

existence of Hardy–Weinberg equilibrium was confirmed ($p = 0.38$). The genotype frequency and the enzyme activity are presented in Table 2. The analysis revealed that the most frequent genotype was the CT (52.1%). Children with the CC genotype had higher levels of serum PON1 activity ($p < 0.05$). The frequency analysis of genotypes CC, CT, and TT among normal weight and overweight children was not different.

For comparing enzyme activity and lipid profile between groups, patients carrying the T allele were grouped into a single category and compared to those homozygous for the C allele. Even after the stratification by nutritional status, serum PON1 activity was higher among patients with the CC genotype ($p < 0.05$). HDL-C serum concentrations were significantly higher in normal weight children, carrying CC genotype, compared to carriers of the T allele. The levels of TC, LDL-C, and TAG were not different between groups, even after stratified by sex ($p > 0.05$) (Table 3).

Discussion

The results of the current study indicated that children with the CC genotype for the PON1 C(−107)T polymorphism had higher serum PON1 activity than carriers of the T allele. In addition, serum concentrations of HDL-C were higher in normal weight children of the CC genotype. The association between the CC genotype and higher serum PON1 activity and HDL-C concentrations in normal weight children creates a protective profile against the development of CVD already in early ages and supports the idea that a favorable environment and genetic predisposition can play a role in disease development.

PON1 arylesterase activity was, as expected, similar for both sexes and also similar to the average PON1 activity for infant populations assessed using phenyl acetate as substrate [12, 17]. Similar results have been reported by other studies [18]. Moreover, in the CHAMACOS Mexican cohort, the authors observed that the average PON1 activity in children raised from age five to seven was similar between boys and girls [19].

The genotypic and allelic frequencies of the studied sample were also similar to those found in Mexican children [19], in contrast to observed in Caucasian adult populations, where the C and T alleles frequencies were close to 50% [8, 20].

Table 2 Serum PON1 activity and genotype distribution in children aged 5–7, Pelotas, RS, Brazil, 2015. ($n = 73$)

PON1 C(−107)T	Genotype		PON1 activity (kU/L)*	
	n	Frequency (%)	Median	p25–75
CC	25	34.2	103.0	83.4–113.9
CT	38	52.1	83.3	66.5–97.2
TT	10	13.7	68.0	63.5–78.7
Total	73	100.0	86.5	66.6–103.6

*Kruskal–Wallis test. $p < 0.01$

Children of the CC genotype had the highest serum PON1 activity. This finding is in agreement with a previous report in children, when it was found that those with the CC genotype had higher PON1 activity than children carrying T allele [9]. In addition, this relationship between the genotype and PON1 activity was greater in normal weight children [21], which is associated with the fact that PON1 arylesterase activity is directly proportional to the protein concentration [22]. PON1 sequencing and haplotype analyses have shown that the C(−107)T PON1 promoter polymorphism is the strongest known predictor of PON1 gene expression and protein levels [20]. Moreover, there is evidence of a molecular mechanism involving C(−107)T PON1 and the binding of the transcription factor Sp1 [6]. This mechanism is consistent with the association between the C allele and higher expression of the PON1 gene, serum protein concentration and serum activity, indicating that the transcription factor binds to its site with more affinity in the variant −107C. In contrast, the −107T reduces the affinity of the Sp1 transcription factor to its binding site, resulting in lower protein concentrations of PON1 for this allele [6].

The association between excess weight in childhood and PON1 SNPs is still poorly investigated [23, 24]. In particular, the association of PON1 C(−107)T and the nutritional status of children have been not studied yet. In our study, we verified that the genotypic distribution was similar between normal weight and overweight children, suggesting that this SNP did not influence the nutritional status.

Many authors have found that there is a reduction in serum PON1 activity in obese children and adolescents compared to those of normal weight [5, 12, 18, 25, 26]. Our results are consistent with others [27, 28] that assessed children of a very similar age group, and found that PON1 activity was higher in obese children than in those of normal weight. Furthermore, studies in adolescents also found similar results [29]. It is possible that the complex nature of obesity, which depends on both genetic and environmental factors, is a confounding factor, resulting in conflicting results about the relationship between the SNP and PON1 activity in children. In this sense, the influence of the gene-environment relation of PON1 activity requires further investigation.

We verified that normal weight children of the CC genotype had HDL-C levels higher than those carrying at least one T allele. These results are in agreement with a study with elderly Italians [30]. One possible interpretation for this effect refers to an increased concentration of PON1 bound to HDL apolipoproteins that may play a role in the metabolism of lipoproteins, improving its

Table 3 Relationship of PON1 C(−107)T with the lipid profile of normal weight and overweight children aged 5–7, Pelotas, RS, Brazil, 2015. ($n = 73$)

		PON1 C(−107)T		*P* value
		CC	CT+TT	
		Median (p25–75)		
PON1 activity(kU/L)[a]	Normal weight	94.6 (72.3–114.8)	68.5 (63.5–86.7)	<0.01
	Overweight	103.6 (100.9–113.0)	94.0 (73.0–102.4)	0.04
TC (mg/dl)[a]	Normal weight	162.0 (141.0–188.0)	165.0 (125.0–175.0)	0.38
	Overweight	165.5 (154.0–183.0)	167.0 (132.0–187.0)	0.48
TAG (mg/dl)[a]	Normal weight	63.0 (52.0–71.0)	54.0 (48.0–86.0)	0.75
	Overweight	78.0 (62.0–85.0)	62.0 (55.0–78.0)	0.40
		Mean (SD)		
HDL-C (mg/dl)[b]	Normal weight	59.8(11.6)	49.0(12.0)	<0.01
	Overweight	49.0(16.6)	50.5(14.6)	0.80
LDL-C (mg/dl)[b]	Normal weight	85.7(28.1)	84.7(31.8)	0.92
	Overweight	101.0(17.9)	89.8(32.9)	0.32

PON1 paraoxonase1, *TC* total cholesterol, *TAG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol
[a]Mann-Whitney test
[b]Student's *t* test

functionality [30]. It indicates that normal weight children of the CC genotype, in addition to increased PON1 activity, also have higher HDL-C levels, suggesting a profile indicator of increased protection against oxidative damage, thus preventing the development of early endothelial injuries.

Recent studies have pointed out that vitamins A, E, and C supplementation have an impact in restoring the decreased paraoxonase activity following different oxidative stress-causing conditions [31]. Moreover, they have verified that, despite the differential response of genetic polymorphisms, some vitamin and antioxidant compounds exert protective action on the PON1 arylesterase activity [31]. The investigation of this important aspect was not possible in our study, since only 6 children, in a total of 73, have used dietary supplements 90 days before the interview.

Finally, it is important to highlight the limitation of the present study. In our analysis, the sample size is appropriate for the study of the genotype distribution but can be considered small for the analyses by subgroup of weight category, something that may limit the extrapolation of our observations to the general population. Surely, it would be interesting to perform a study with a higher sample size, in order to clarify in more detail the association between the PON1 polymorphism and nutritional status over the enzyme activity.

Conclusion

We conclude that PON1 C(−107)T polymorphism is associated with higher serum enzyme activity in children, as observed previously in adults. In addition, this polymorphism also shows association to higher HDL levels and serum PON1 arylesterase activity in the normal weight children studied.

Abbreviations

BMI: Body mass index; *Bsr*BI: Restriction enzyme digestion; CVD: Cardiovascular disease; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; PCR: Polymerase chain reaction; PON1: Paraoxonase 1; SD: Standard deviation; SNP: Single nucleotide polymorphism; TAG: Triglycerides

Acknowledgments

We warmly thank Pedro Silveira for helping with the samples genotyping. We also thank Caroline Maciel and Tainá Sigales for helping with the data collection and the enzyme activity analysis. We thank Dr. Denise Mota for critical contributions in the project as well as we thank the pediatric ambulatory staff of the Universidade Federal de Pelotas for helping with the child recruitment.

Funding

This work was supported by CNPq and CAPES.

Authors' contributions

UG was responsible for performing all stages of the study. MLC supported the statistical analysis and writing of the article. BCC supported the paraoxonase-1 genetic analysis and collaborated with the revision of the final version of the article. SA supported the paraoxonase-1 activity measurement, genetic analysis, and collaborated with the revision of the final version of the article. VSC performed the study's conception, supervised all stages of research, and performed the writing of the final version of the article. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interest.

Consent for publication

Not applicable.

Ethics approval and consent to participate
The study protocols were approved by the Ethics Committee on Human Research of College of Medicine/Universidade Federal de Pelotas (CAAE number: 25869013.8.0000.5317/Report number: 654.939)

Author details
[1]Department of Nutrition, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. [2]Food for Health Science Centre, Lund University, Lund, Sweden. [3]Departamento de Nutrição, Faculdade de Nutrição, Universidade Federal de Pelotas, Rua Gomes Carneiro, 1, Pelotas, RS CEP 96010-900, Brazil.

Received: 2 May 2016 Accepted: 21 November 2016
Published online: 15 December 2016

References

1. Sahebkar, A., Hernández-Aguilera, A., Abelló, D., Sancho, E., Camps, J., Joven, J. Systematic review and meta-analysis deciphering the impact of fibrates on paraoxonase-1 status. Metabolism-Clinical and Experimental 65, 609–622, 2016. DOI: http://dx.doi.org/10.1016/j.metabol.2016.01.002
2. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol. 2001;21:473–80.
3. Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, et al. Low paraoxonase activity predicts coronary events in the caerphilly prospective study. Circulation. 2003;107:2775–9.
4. Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics. 1996;33:498–507.
5. Ferre N, Feliu A, Garcia-Heredia A, Marsillach J, Paris N, et al. Impaired paraoxonase-1 status in obese children. Relationships with insulin resistance and metabolic syndrome. Clin Biochem. 2013;46:1830–6.
6. Deakin S, Leviev I, Brulhart-Meynet MC, James RW. Paraoxonase-1 promoter haplotypes and serum paraoxonase: a predominant role for polymorphic position - 107, implicating the Sp1 transcription factor. Biochem J. 2003;372:643–9.
7. Schrader C, Rimbach G. Determinants of paraoxonase 1 status: genes, drugs and nutrition. Curr Med Chem. 2011;18:5624–43.
8. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, et al. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet. 2001;68:1428–36.
9. Huen K, Harley K, Bradman A, Eskenazi B, Holland N. Longitudinal changes in PON1 enzymatic activities in Mexican-American mothers and children with different genotypes and haplotypes. Toxicol Appl Pharmacol. 2010;244:181–9.
10. Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. Biochem Pharmacol. 2005;69:541–50.
11. Sociedade Brasileira de Pediatria - SBP. Departamento Científico de Nutrologia. Obesidade na infância e adolescência – Manual de Orientação. 2ª Ed, pp 142, 2012.
12. Koncsos P, Seres I, Harangi M, Illyes I, Jozsa L, et al. Human paraoxonase-1 activity in childhood obesity and its relation to leptin and adiponectin levels. Pediatr Res. 2010;67:309–13.
13. Najafi M, Gohari LH, Firoozrai M. Paraoxonase 1 gene promoter polymorphisms are associated with the extent of stenosis in coronary arteries. Thromb Res. 2009;123:503–10.
14. Campo S, Sardo MA, Trimarchi G, Bonaiuto M, Castaldo M, et al. The paraoxonase promoter polymorphism (−107)T>C is not associated with carotid intima-media thickness in Sicilian hypercholesterolemic patients. Clin Biochem. 2004;37:388–94.
15. WHO. Organization WH Anthro plus for personal computers: software for assessing growth and development of the world's children. Geneva: WHO; 2007.
16. Cardiologia SBd, Metabologia SBdEe. I Diretriz de Prevenção da Aterosclerose na Infância e na Adolescência. Arq Bras Cardiol. 2005;85:3–36.
17. Sumegova K, Nagyova Z, Waczulikova I, Zitnanova I, Durackova Z. Activity of paraoxonase 1 and lipid profile in healthy children. Physiol Res. 2007;56:351–7.
18. Cayir Y, Cayir A, Turan MI, Kurt N, Kara M, et al. Antioxidant status in blood of obese children: the relation between trace elements, paraoxonase, and arylesterase values. Biol Trace Elem Res. 160, 155–160, 2014
19. Huen K, Harley K, Brooks J, Hubbard A, Bradman A, et al. Developmental changes in PON1 enzyme activity in young children and effects of PON1 polymorphisms. Environ Health Perspect. 2009;117:1632–8.
20. Suehiro T, Nakamura T, Inoue M, Shiinoki T, Ikeda Y, et al. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. Atherosclerosis. 2000;150:295–8.
21. Draganov DI, La Du BN. Pharmacogenetics of paraoxonases: a brief review. Naunyn Schmiedebergs Arch Pharmacol. 2004;369:78–88.
22. Huen K, Yousefi P, Street K, Eskenazi B, Holland N. PON1 as a model for integration of genetic, epigenetic, and expression data on candidate susceptibility genes. Environ Epigenetics. 2015;1:1–11.
23. Huen K, Harley K, Beckman K, Eskenazi B, Holland N. Associations of PON1 and genetic ancestry with obesity in early childhood. PLoS One. 2013;8:e62565.
24. Ruperez AI, Lopez-Guarnido O, Gil F, Olza J, Gil-Campos M, et al. Paraoxonase 1 activities and genetic variation in childhood obesity. Br J Nutr. 2013;110:1639–47.
25. Krzystek-Korpacka M, Patryn E, Hotowy K, Czapinska E, Majda J, et al. Paraoxonase (PON)-1 activity in overweight and obese children and adolescents: association with obesity-related inflammation and oxidative stress. Adv Clin Exp Med. 2013;22:229–36.
26. Zaki ME, El-Bassyouni H, Kamal S, El-Gammal M, Youness E. Association of serum paraoxonase enzyme activity and oxidative stress markers with dyslipidemia in obese adolescents. Indian J Endocrinol Metab. 2014;18:340–4.
27. Garcés C, López-Simón L, Rubio R, Benavente M, Cano B, et al. Análisis de la actividad paraoxonasa (PON1) y de los polimorfismos PON1 192 y PON1 55 en la población prepuberal del Estudio Cuatro Provincias*. Clínica e investigación en arteriosclerosis. 2007;19:287–92.
28. Gonzalez V, Huen K, Venkat S, Pratt K, Xiang P, et al. Cholinesterase and paraoxonase (PON1) enzyme activities in Mexican-American mothers and children from an agricultural community. J Expo Sci Environ Epidemiol. 2012;22:641–8.
29. Eren E, Abuhandan M, Solmaz A, Taskin A. Serum paraoxonase/arylesterase activity and oxidative stress status in children with metabolic syndrome. J Clin Res Pediatr Endocrinol. 2014;6:163–8.
30. Campo S, Sardo MA, Trimarchi G, Bonaiuto M, Fontana L, et al. Association between serum paraoxonase (PON1) gene promoter T(−107)C polymorphism, PON1 activity and HDL levels in healthy Sicilian octogenarians. Exp Gerontol. 2004;39:1089–94.
31. Lou-Bonafonte JM, Gabás-Rivera C, Navarro MA, et al. PON1 and Mediterranean diet. Nutrients. 2015;7:4068–92.